Project-2

Revised

RESEARCH PROPOSAL

3

1.	**Project Title**	:	Comparison of tiger (Panthera tigris) population estimated using noninvasive techniques of pug mark, camera trap and DNA based analysis of hair and scat in Ranthambhore Tiger Reserve. **Phase I :** A pilot study to standardize protocols for identifying free ranging individual tigers
2.	**Name and Designation of the Principal Investigators**	:	Dr. S.P. Goyal, Sr. Reader
3.	**Co-Investigators**		Dr. K. Sankar, Sr. Reader Shri Q. Qureshi, Reader
4.	**Advisors**	:	Shri V.B. Sawarkar, Profesor & Dean, Faculty of Wildlife Science Dr. A.J.T. Johnsingh, Professor and Head, Division of Animal Ecology and Conservation Biology
5.	**Name of the Institution in which the project will be carried out**	:	Wildlife Institute of India, Dehra Dun
6.	**Name of the other Institution likely to be involved**	:	DNA Typing Unit, Central Forensic Science Laboratory, Kolkata Foreign collaborators specialized in using noninvasive techniques in estimating carnivore population* Forest Department, Rajasthan Other institutions/Departments if required
7.	**Time required for the commencement of the project on receipt of approval**	:	Six to eight months after final approval
8.	**Duration of the project**	:	Eighteen months

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9. Total amount of the financial layout for Eighteen months:

1.	Fellowship and wages	453400
2.	Purchase of equipment	340000
3.	Purchase of Gypsy, POL & Maintenance	660000
4.	DNA based work (chemicals, plastic wares, primers etc)	200000
5.	Travel and DA	70000
6.	Base camp establishment	123000
7.	Report Writing	15000
	Grand Total	1861400

Documents enclosed:

Statement – I	Abstract
Statement – II	Detailed Project Proposal
Statement – III	Project Budget Estimate

Signature of the Principal Investigator

Signature of the Chairman, IRAC

Signature of Director, WII

- Dr. Rober Wielgus, Population Ecologist and Dr. Lisette Waits, Molecular Ecologist of Washington State University and the University of Idaho respectively are collaborated with the institute's project on Leopard in Garhwal. We would explore possibility of their collaboration on this project as well as other scientists. Funds will be raised by the collaborators to meet their expanses during their visit to India.

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STATEMENT – I

Reliable data on the distribution and abundance of a number of carnivore species have always been problematic being the species are secretive, elusive or highly dispersed. The conservation of tiger (Panthera tigris) has been always a global issue being charismatic and flagship species due to the top of the food chain and always prone to poaching. Estimating numbers reliably has been one of the most crucial aspects for the wildlife mangers throughout its range. Zielinski and Kucera (1995) have reviewed various methods evolved for survey and estimating carnivore species. Individuals identified based on pugmark, has been in use for almost three decades in India for knowing tiger numbers.. Camera trap has also recently been used to estimate tiger numbers. Noninvasive DNA based techniques have recently been standardized for estimating population using remotely collected hair and scat in number of elusive species (Foran et al., 1997; Kohn et al., 1999; Mace et al., 2000; Pearse et al., 2001). But there is no study so far exists to compare estimates of tiger determined using various techniques in a given area to come out appropriate tiger population monitoring protocols that are feasible, reliable, repeatable, cost effective and statistically robust at a variety of scales.

First time, we envisaged to undertake a study to determine and compare tiger numbers estimated using baited track plot stations for obtaining pugmark, remote photographic bait station (camera trap) and non-invasive DNA based techniques using scat and remotely collected hair. A pilot study of eighteen months under Phase –I has been planned to standardize protocols and sampling design for identifying free ranging individual tigers based on pug mark, camera trap and DNA techniques. Intensive study area (ISA) of around 50 sq.km in Ranthambhore Tiger reserve (RTR) will be selected. Track plots (TP) popularly used for carnivore survey will be randomly placed on preferred tiger trails in ISA having uniform soil depths (ca. 1 cm) and will be baited with a Carnivore Survey Disc (CSD) or Cat lure to attract surrounding tiger. Pugmarks obtained on each TP will be traced as well as photographed using digital camera following standard protocols. Multivariate statistics will be used to identify individuals on various pugmark measurements determined using Scan Pro–Software. We also planned to determine number of track plots needed, validate tiger identity determined

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based on pugmarks using photo identity and is there any saturation level in pugmark measurements in relation to tiger numbers. Standard Camera Trap will be placed on randomly selected 10 track plots and individuals photographed will be identified based on stripe patterns. DNA based micro-satellite techniques developed recently to know population-using scat by Ernest *et al.* (2000) and hair collected remotely using baited hair snares (hair combs) (Foran *et al.*, 1997) will be tried in ISA. Opportunistic scats will also be collected from ISA. Protocols will be standardized for extracting DNA from scats and hair. We will use PCR techniques employing at least 12 microsatellite loci for determining polymorphic satellites for individuals as studied on mountain lion (Ernest *et al.* (2000). Based on standardized protocols for all techniques, a Phase –II proposal will be planned for comparing tiger populations estimated based on three techniques.

Planned study is for eighteen months and estimated budget is as follows:

1.	Fellowship and wages	453400
2.	Purchase of equipment	340000
3.	Purchase of Gypsy, POL & Maintenance	660000
4.	DNA based work (chemicals, plastic wares, primers etc)	200000
5.	Travel and DA	70000
6.	Base camp establishment, misc., contingency and others	123000
7.	Report Writing	15000
	Grand Total (Rs.)	1861400

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STATEMENT – I I

INTRODUCTION:

The conservation of tiger (Panthera tigris) has been always a global issue being charismatic and flagship species due to the top of the food chain and always prone to poaching. Estimating numbers reliably have been one of the most crucial aspects for the wildlife mangers throughout its range though various attempts have been made.

Among carnivore studies, one of the crucial management and conservation issues has to know distribution, land-tenure system and abundance or population size. Estimation of population size has been central focus for wildlife researchers as well as managers. Option for estimating carnivores population size are few and often requires specific circumstances or detailed assumption which are often difficult to meet. Distribution and relative abundance estimation of smaller carnivores have been major focus among all carnivore species. A number of research articles have been published determining based on track and remote camera system been for estimating carnivores abundance and has been reviewed by Zielinski and Kucera (1995).

One of the widely used methods for estimating population is mark-recapture, in which an initial population sample is captured, marked and released. The population is then resampled during various sessions. The ratio of newly captured animals to recaptures is then used to compute a population estimate (White *et al.*, 1987). Important thing in this method is to mark captured individuals. In wildlife research, various forms of ear tags, colored bands, neck collars, radio transmitters, and natural markings to identify and individual's track under field conditions (Nietfeld *et al.*, 1994). Each method has advantages and limitations because artificial markers are either lost or natural markers are difficult to use to identify due to changes in their patterns. Under such prevailing conditions, the ideal mark would be non-invasive, highly visible, clearly read, inexpensive and permanent.

Tracks (pugmarks) have very often been used for understanding population and demography of carnivores species which are sparsely distributed, cryptic, elusive, nocturnal and solitary by identifying individuals and understanding land tenure system.

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Pugmark technique developed by S.R. Choudhury (Choudhury, 1972) is being in practice over years for determining tiger numbers in India. The technique has been refined (Panwar, 1979; Sawarkar, 1990; Sharma, 2001) and recently been compiled (Singh, 1999). Major assumption is that pugmark of each individual tiger is different from other but it was never validated under field conditions to determine accuracy and precision. This leads to controversy on the estimates of tiger reported so far (Karanth, 1995). It is well known that different type of pugmarks are produced while walking by same individual on different soil types with in its home range. Despite the usefulness of pugmark methodologies, use of quantitative approach has been slow to develop. Among large carnivores, pug mark characteristics and measurements have been used to determine sex and individuals tigers (*Panthera tigris*) (Gore *et al.*, 1993; Karanth, 1995; Sharma, 2001) and leopards (*Panthera pardus*) (Miththapala *et al.*, 1989) and several African carnivores (Stander et al., 1997). Among quantitative approaches in characterizing large carnivores tracks, Smallwood & Fitzhugh (1993) used multi-group discriminate analysis to separate track of individuals mountain lions (*Felis concolor*) in three regions of California. It was not very clear that whether the approach can distinguish tracks from an unknown numbers of individuals occupying the same geographical area. Grigione *et al.* (1999) attempted to refine the methods developed by Smallwood & Fitzhugh (1993) by monitoring and following tracks of ten radio collared mountain lions in the field and concluded that track sets had both correct and incorrect "groupings" and these were sensitive to the type of substrate, no. of tracks in set and time of day it was photographed. More such studies are needed to validate accuracy and precision in identifying individual's pugmark by incorporating known individuals under free ranging field conditions used by resident as well by transit animals. It is not correct to say that only tracks of one or two individuals will be seen in a area as Franklin et al. (1999) found six and fifteen resident and non resident tigers respectively while studying in a study area of 160 sq. km of Way Kambas National Park, Indonesia.

Among various devices used for knowing presence or absence of species, self-activating camera systems have been used since at least the early 1900s (Gregory, 1927) for various purposes in wildlife studies such as species using particular food sources (Gysel and Davis, 1956); watering hole (Davis and Bleich, 1980); identification of

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species preying on bird nests (Savage and Seibert 1988; Major, 1991); scavenging carcasses and estimation of consumption rates (Wilton 1989); monitoring animals use of caves (Winkler and Adams, 1968); or highways or underpasses (Foster,1992); investigation of activity patterns (Osterberg, 1962; Buckner, 1964); quantification of behavior (Diem *et al.*, 1974, Harris, 1982); enumeration of individuals. Raphael (1993) determined number of visits to baited camera stations and used data to estimate an index of species abundance where as Jacobson *et al.* (1997) estimated white-tailed deer population using infrared triggered cameras. Other studies have used in determining population size and composition by applying mark-recapture (or resight or resample) abundance estimators to photographic sampling of marked populations (i.e. determination of the proportion of photographed animals that were tagged or collared) (Jaeger *et al.*, 1991; Dusek and Mace, 1991). Mace *et al.*, 1994 have estimated Grizzly bear population using camera sightings. Over the years remotely triggered camera has been more and more use in wildlife studies for determining population size or other ecological aspects of elusive, secretive and nocturnal species such as tiger (Karanth, 1995; Franklin *et al.*, 1999).

Very recently, genetic 'tag' in the form of microsatellite genotypes have the potential to meet several of these criteria needed for molecular tracking and identifying individuals and advantages in technology are making DNA methods accessible at the field level (Parker *et al.*, 1998). Thus genetic tags can replace conventional marks in these studies if the tags reliably identify individuals during a series of sampling session. Use of such tags or DNA based mark-recapture population estimation have been widely demonstrated in census of black bear, brown bear, grizzly bear, martens and North Atlantic humpback whales (Palsboll *et al.*, 1997).

Recent developments in techniques to estimate population size of bear species have included use of remote hair capture to sample population, DNA analysis to identify individuals and mark-recapture modeling to estimate population size (Woods *et al.* 1996; 1999; Mowat and Strobeck, 2000). These techniques appear to provide an accurate, less costly, less invasive alternative to population estimates derived from intensive capture and radio collaring efforts (i.e. McLellan, 1989; Stirling *et al.*, 1997).

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Whole blood and tissue biopsies obtained from captured animals have been routinely used as source of DNA. Several recent studies have obtained DNA from free ranging animals using alternative tissue sources like skin samples from hump-back whales (Palsboll *et al.*, 1997), feces from brown bears (Hoss *et al.*, 1992), black bears (Wasser *et al*, 1997) and seals (Reed *et al.*, 1997) and hair from American marten (Foran et al., 1997), brown bears (Taberlet *et al.*, 1993) and chimpanzees (Morin *et al.*, 1994). Roots of mammalian hair contain sufficient DNA for analysis when genetic material at specific loci is amplified using the Polymer Chain Reaction (Higuchi *et al.*, 1988). For free ranging bears, hair is an alternative DNA source because bears frequently leave hair on rub tress, in beds and at foraging sites(Taberlet and Bouret, 1992). Because bears are readily attracted by scent lures, methods to obtain hair samples from free ranging bears permits systematic sampling regimes necessary for many ecological studies such as animal census.

Woods *et al.* (1999) have developed method for collection of genetic materials from free ranging black and brown bears and tested three methods for collection of hair samples viz. barbed-wire enclosure, cubby with barbed-wire, cubby with wire and dog brush. Among these, they found that barbed-wire enclosure hair trap was supervior.

Widely employed markers include proteins, nuclear DNA (nDNA) and mitochnodrial DNA (mtDNA). nDNA (dploid) contains two copies of each gene or markers (one from the individual's mother and one from father) and it is very useful in assigning individual identity, sex, relationships to near relatives, and population structure. mtDNA is inherited from mothers only and is useful in studying evolutionary relationships, female population structure and gene flow, and identifying species. The PCR allows minute quantities of DNA to be replicated many times and supplies sufficient quantities of DNA for analysis. Particular regions of DNA (loci) are isolated with appropriate primers, amplified using PCR, separated on an acrylamide or agrarose gel and visualized with radioactivity, flourescenes, or ethidium bromide. Microsatellites are examples of highly variable loci with alleles that differ by the numbers repeated sequences units such as CA. Microsatelllite loci, consisting of tandem repeats of short core sequence of one to five nucleotides, are particularly useful for population genetic studies of natural population (Bruford and Wayne, 1993; Queller *et al.*, 1993) as they are

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abundant and widely dispersed in eukayotic genomes (Tautz *et al.*, 1986; Tautz, 1989; Weber and May, 1989), have high mutation rate and are highly polymorphic (Dallas 1992; Dietrich *et al.*, 1992; Ellegren, 1992).

Among genetic studies, it is first important thing to demonstrate that the variability of alleles at the available loci is sufficient in the study population to be useful in identifying individuals. To certain extent, increasing the number of loci used can offset lower levels of variation. In studies dealing in identifying individuals from randomly collected sample, it is important to address question of certainty. Best way, the problem has been approached by determining heterozygosity which is a measure of genetic diversity at a particular set of loci in a population. Paetkau *et al.* (1998) measured variation in genetic diversity across the range of North American brown bear and concluded that it is necessary to know the allele frequency distribution within in a population in order to calculate the number of loci that needed for a particular level of certainty in declaring matches between samples. When samples differ from each other by one or more alleles they come from different individuals. Statistical methods were described which allow a probability statistics to be assigned to each sample (Waits et al., 2000). They evaluated the accuracy of probability of identity (PID) estimation by comparing the observed and expected PID using a large nuclear DNA mircosatellite data sets from three endangered species: the Grey wolf, the brown bear and Australian northern hairy-nosed wombat. To avoid biases, they introduce an equation for PID between sibs. Woods *et. al.* (1999) used six microstaellite loci in black and brown bears and developed match probability functions and suggested to use formula involving presence of sibling in the sample (Psib) to each sample (match criteria at Psib<0.05) and illustrated to use microsatellite genotypes can be used as genetic tags in mark-capture bear censuses. Woods et al. (1999) suggested that 4 to 6 loci are needed to achieve standard genotype within our study population but suggested that match must be made on an individual basis, not simply by calculating a mean expected probability of exclusion for the study population.. Preliminary work Woods and McLellan (1995) on black and grizzly bears observed a high degree of variability and demonstrated that the probability of incorrectly identifying individuals in random samples based on eight loci was estimated to be 1/1.5 trillion for black bears and 1/5.7 billion for grizzly bears. A P(ID) of

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less than approximately 0.01 will be necessary for studies
estimation (Mills et al.

using mark-recapture models. Population size has been estimated using non-invasive DNA based techniques for Atlantic humpback whale (Posoboll et al., 1997) and coyote (Kohn *et al.*, 1998) and grizzly bear (Wood *et al.*, 1999; Mowat and Strobeck, 2000).

Thus we planned to estimate and compare tiger population by utilizing all existing methods used for population estimation of large carnivores such as pugmark, camera trap, and DNA based techniques using scat and remotely collected hair samples. Under Phase – I, we aimed to standardize protocols and sampling design for identifying free ranging individual tigers using pugmark, camera trap and DNA techniques.

OBJECTIVES:

Under the proposed Phase-I project, we aimed to

1. Determine suitable sampling design, sample size and level of saturation in pug mark characteristics, if any, for identifying free ranging individual tigers based on pugmarks and camera traps,
2. Validate identity of individual tiger determined based on pugmark using photo identity,
3. Standardize techniques for remotely collection of tiger hair using from hair snares,
4. Develop protocols for extracting DNA from hair and scat and determine number of polymorphic satellite needed for identifying individual tigers, and
5. Prepare Phase-*II* proposal based on standardized protocols for comparing tiger population estimated by three methods.

STUDY AREA:

The present study will be carried out in Ranthambhore National Park (RNP) (25° 54'N-26° 12'N latitude and 76° 22'E-76° 39'E longitude) of 392 sq.km in Sawai Madhopur district of Rajasthan of which 274 sq.km is core area. RNP mainly covers Aravali and Vindhya hill ranges. The vegetation of RNP is a typical representative of dry-deciduous *Anogeissus pendula* forest. Champion and Seth (1968), considered the

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vegetation of RNP as being comprised of (i) Tropical dry deciduous forest and (ii) Tropical Thorn Forest. Apart from *Anogeissus*, the other species commonly found are *Sterculia urens*, *Boswellia serrata*, *Acacia leucophloea*, *Cassia fistula*, *Butea monosperma*, *Diospyros melanosylon*, *Cordia myxa*, *Mitragyna parviflora* and *Syzigium cumini*.

Ranthambore is characterized by subtropical dry climate with four distinct seasons viz. summer (March-June), monsoon (July to August), post-monsoon (September to October) and winter (November to February). The average rainfall is about 800 mm and most of which is received during July to September. RNP supports five species of wild ungulates, viz. chital, sambar, nilgai, chinkara and wild pig. The large predators are tiger (*Panthera tigris*) and leopard (*Panthera pardus*), apart from which, striped hyaena, jungle cat, caracal, common palm civet, are also found.

An 'Intensive Study Area' (ISA) of around 50 sq.km will be selected within core area of RNP having two major perennial water sources (Rajbagh and Padam Talav) and having fairly good concentration of tiger.

METHODS:

I. Recording Pugmark to identify individuals:

One of the major inherent biases in identifying individuals from the pugmark was large variation in pugmark characteristics of the same individuals due to varying topogrphy and soil types and texture and amount of moisture (Karanth, 1995; Singh, 1999; Melissa et al., 1999) and time of the day while photographing (Melissa et al., 1999). Over the years, attempt have been made to refine the techniques and use quantitative and objective approaches for identifying individuals or sex (Gore *et al.*, 1993; Melissa *et al.*, 1999; Sharma, 2001). Based on my personal experience while working on leopard, tracks obtained on a hard surface having chowk powder of 1 cm thickness provided track of much more consistent and uniform in measurements. We intend to use the standard protocols suggested for the pugmark census (Choudhury, 1972; Panwar, 1979; Sawarkar, 1987, 1990; Singh, 1999; Sharma, 2001) with some of the modifications/refinement as suggested by Melissa *et al.* (1999) while recording or

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photographing pugmark with respect to type of substrate, time of the day when it was photographed and number of tracks in a set.

Around forty permanent track plots will be randomly placed on tiger trails in the ISA. We intend to trace the hind left or right pugmark using the standard protocols as described (Panwar, 1979; Sawarkar, 1987, 1990; Singh, 1999) using glass tiger tracer. Same pugmark traced will also be photographed using digital camera taking all precautions described by Melissa *et al.* (1999) and Sharma (2001) to reduce the chances of error due to photographing. Tracing as well as digital photographs of the pugmarks will be transferred to the computer for further computational analysis. Various linear and non linear, angle and area measurements as used by Sharma (2001) will be done for each pugmark using Scan Pro Software. Multivariate statistics including cluster analysis as well as discriminate analysis (Grigione *et al.*, 1999) will be employed to pugmark tracing/images of different individuals. Such analysis would enable us to establish individual identity. Track plots will be monitored continuously at least for six months which would enable us to determine how many days and track plots are needed to monitor for identifying tiger individuals present in ISA. We will also determine level of saturation in pugmark characteristics, if any in reference to tiger numbers and validate identity of tiger determined based on pugmarks using photo identity.

2. Camera Trap : Each Camera trap system consisted of 35 mm fully automatic camera with auto flash, a passive infrared (PIR) sensor which triggers camera whenever any animal passes in front of PIR sensor. Photo identity of each tiger will be prepared based on stripes patterns as it is well documented that strip patterns of each tiger is unique and genetically determined. Ten randomly selected track plots of the selected twenty for pug mark will be used for placing camera trap.

Tiger photographs were separated as individuals based on the sex wherever it is possible to identify, body stripe patterns and other morphological distinguishing features. We will establish a reference database of quality tiger photographs. We will also determine sample size needed for identifying individuals using camera trap in ISA.

3. Non-invasive DNA based techniques:

Non-invasive DNA based techniques for estimating population involve the (i) the collection of biological materials (scat/hair/tissue) suitable for DNA extraction, and (ii)

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determining genetic typing using microsatellite. We intend to employ DNA based techniques to identify individuals using scat (fecal) and remotely collected hair of tiger.

a. **Scat Collection:** Fresh scat (feces) determined based on moisture and disintegration, will be collected *ad libitum* walking along various trails within ISA. The entire sample was mixed thoroughly as described by Wasser *et al.* (1997). and a 2 g portion stored in 25 ml vial capacity containing silica gel (4 g silica/g faces, silica gel beads Type II, 1/8" Sigma Chemical Company, St. Louis, MO, USA) until processed. We will prepare at least four such vials for each scat found. Each vial will be duly marked place along with GPS location and any other secondary information.

b. **Remotely (non-invasive) collection of tiger hair samples:** Hair follicles contain enough DNA for further analysis and techniques have been standardized for remotely collecting of hair by using mechanical devices (e.g. barbed wire, saw blades) (Raphael, 1994) and glue hair snares (available commercially as glue traps used to entangle mice and rats) (Foran *et al.*, 1997). We planned to try various hair snares like hair combs or any other materials. Hair snare will be attached to a tree trunk opposite to the camera trap on selected track plot and will be baited in center with "Cat Lure" to attract the animal. Hair snares will be regularly checked daily. Hair will be removed from hair snare and placed in polyethylene vial half filled with silica gel (silica gel beads Type II, 1/8" Sigma Chemical Company, St. Louis, MO, USA) as suggested by Foran *et al.* (1997) and they found that such storage retained high molecular weight DNA even after several months at room temperature as well as at –20°C or 37 °C. We will follow the protocols as described by Foran *et al.* (1997) for removing hair from glue trap without degrading DNA. Before processing for DNA extraction, the collected hair samples were examined microscopically for their cuticular patterns for ascertaining that collected hairs are of tiger. Once confirmed, hair samples will be used DNA analysis

c. **DNA extraction from scat and hair samples:**

We will follow DNA extraction from scat and hair follicles as described by Taberlet *et al.* (1997). DNA extraction from hair collected for tiger will be carried out using the Chelex method as described by Walsh et al (1991). DNA extraction from scat will be done based on silica method (Boom *et al.*, 1990; Hoss *et al.*, 1992; Hoss and

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Paabo, 1993). Nearly three to five extractions will be carried out for each feces and extracted DNA will be stored at –20oC till further analysis. Blanks will be run along with DNA extraction to determine whether any contamination with exogenous DNA has occurred during procedures.

d. Determining polymorphic microsatellite for individual identifications:

Twelve domestic cat microstaellite primers (Menotti-Raymond & O'Brien ,1995; Menotti-Raymond *et al.*, 1997; 1999) viz. Fca8, Fca23, Fca 35, Fca43, Fca77, Fca78, Fca90, Fca96, Fca126, and Fca132, used for mountain lion and other cat species by Ernest *et al.*, 2000, will be used for genotyping. Genotypes will be then classified as heterozygotes if two DNA fragments observed and homozygotes if only a single fragment is observed. Probabilities of genotype identity for a given locus will be calculated using the following formula given by Paetkau *et al.* (1995):

$$I = \sum p_i^4 + \sum\sum (2\, p_i\, p_j)^2$$

where p_i and p_j are the frequencies of the ith and jth alleles for a particular locus in the population. Overall genotype identity is then calculated as the product of the identity probabilities over all loci.

One of the major problems while genotyping individuals from DNA extracted from scat is of allele drop out and leading to an incorrect genotyping of individuals. The problem has been seen while genotyping Bonobo (*Pan pygmeus*) feces (Gerloff *et al.*, 1995) and same problem might come while working on tiger scat. Therefore multiple tubes procedures of Taberlet *et al.* (1995) will be followed for reliable genotyping individuals. We will determine highly polymorphic microsatellite suitable for tigers of RTR for genotyping and standardize protocols for Phase-II study.

Justifications:

Carnivore species specially on top of the food chain like tiger are always occurred in low density than the other species of the food web occupying lower niches. Tigers being very sensitive to habitat change and prone to poaching for bones and skin, one of the important task for better management of elusive carnivore species is to know their

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reliable distribution and population. Over three decades, a number of techniques have been evolved for monitoring carnivores based on pugmark, Camera Trap and using noninvasive DNA techniques to understand land tenure system and determine population. Barring a few attempts, no study has so far been undertaken to compare existing all techniques for monitoring and estimation population of carnivore species in a given area.

Proposed project is first attempt in India to compare population estimation of tiger determined from various methods in Ranthambhore Tiger Reserve to address this issue and to come out appropriate protocols which are practical, suitable at variety of scales and cost effective to estimate tiger number.

Under Phase-I study, we have envisaged to standardize protocols for determining individual tigers using pugmark, camera trap and DNA based techniques. Therefore, two researchers having background of Wildlife Biology and Molecular Ecology are necessary and have been proposed. Two field assistants are needed for regular monitoring of track plots, camera trap and hair snares..

Four wheel vehicle is needed to operate ten camera traps, monitor track plots and hair snares and collection of scats. Photo-identity of tigers will be prepared using remotely triggered cameras. Therefore, planned to use at least ten camera trap at a time and thus have been proposed for purchase. Digital camera and computer with scanner and printer have been proposed for purchase to use for capturing pugmark images from digital camera and transfer directly to computer to undertake various measurements for identifying individuals.

Responsibilities of Principal and Co-investigators:

1 Dr. S.P. Goyal, Principal Investigator will be overall in-charge for co-ordination for smooth functioning of project and provide inputs at all the levels.

2 Dr. K.Sankar, Co-investigator will be mainly responsible for providing inputs in collection of data using pug mark and camera trap techniques.

3 Shri Q. Qureshi, Co-investigator will be mainly responsible for providing inputs in sampling design and statistical analysis of data collected using various techniques.

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Work Plan

Activities	Month								
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18
Selection of JRFs and review of literature									
Purchase of equipments									
Standardizing protocols for identifying free ranging tigers based on pug marks and camera traps									
Developing methodology for remote collection of tiger hair									
Standardizing protocols for DNA extraction from hair and scats									
Determining polymorphic microsatellites to identify individuals									
Report writing									
Submission of Phase-II project									

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Research project "Comparison of tiger (Panthera tigris) population estimated using non-invasive technique of pug mark, camera trap and DNA based analysis of hair and scat in Nallamalai tiger reserve forest-I"

Statement – III

Project Budget

S.No.	Project Head	Budget in Rs. for 18 months
1.	**Fellowship and wages**	
1.2	One Junior Research Fellow for pugmark and camera trap (@Rs:8000.00/month	144000
1.2	One Junior Research Fellow for DNA techniques (@Rs.8000.00 /month	144000
1.3	Two Field Assistants @ Rs.2400/month	86400
1.4	One Lab Assistant/TA @Rs.4000/month	72000
1.5	Hiring of Casual laborers	7000
2.	**Purchase of equipment**	
2.2	Camera traps	150000
2.3	Carnivore survey disc, cat lure and others	30000
2.6	GPS (Two)	15000
2.7	Digital Camera	45000
2.8	Computer with scanner and printer	100000
3.	**Purchase of Gypsy, POL & Maintenance**	430000
4.	**DNA based work (chemicals, plastic wares, primers etc)**	200000
5.	**Travel and DA**	
5.1	Travel expense for collaborators	30000
5.2	Travel expense for researchers and field assistants	20000
5.3	Training	20000
6.	**Base camp establishment**	
6.1	Base camp setting	20000
6.2	Accommodation charges	18000
6.3	Field dress allowance for researcher	5000
6.4	Film purchase and development	30000
6.5	Misc. & Contingency	50000
7.	**Report Writing**	15000
	Summary	
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Research/20 proposal.pdf